IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Guilherme L. Indig

Serial No.: 09/753,472 Docket No.: 032026-0667

Filed: January 3, 2001 Examiner: J. D. Goldberg

For: **USE OF CRYSTAL VIOLET AS PHOTOCHEMOTHERAPEUTIC AGENT**

DECLARATION OF GUILHERME L. INDIG

I, Guilherme L. Indig, do hereby declare and state as follows:

1. I am a citizen of the United States of America, residing at 6618 Montclair Lane, Madison, WI 53711.

2. I am an Assistant Professor of Pharmacy at the University of Wisconsin-Madison.

3. I am the inventor of U.S. Application Serial No. 09/753,472 (hereinafter "the Application").

4. My curriculum vitae is attached hereto as exhibit A.

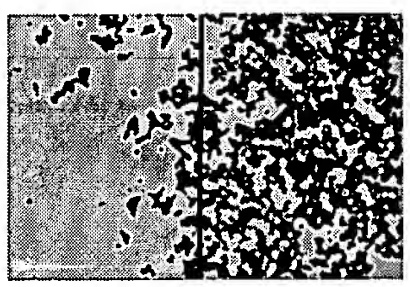
5. I have reviewed the Office Action, mailed May 24, 2002, and the prior art cited therein (hereinafter "the Office Action").

6. The Office Action stated that the specification, while being enabling for the specific cancers disclosed, does not provide enablement for the term "cancer cells" in claims 6-10 of the Application.

7. Figure 1, shown below, shows data obtained by Irawati K. Kandela, a graduate student in my research group, under my direct supervision. In Figure 1(a), the right half of the panel shows a colony of HT-29 human colon adenocarcinoma cells that have been

exposed to crystal violet but not fluorescent light. The left half of the panel in Figure 1(a) shows a colony of HT-29 human colon adenocarcinoma cells that have been exposed to crystal violet and fluorescent light. The data of Figure 1(a) demonstrates that crystal violet has a phototoxic effect on HT-29 human colon adenocarcinoma cells when irradiated with fluorescent light of an appropriate wavelength. In Figure 1(b), the right half of the panel shows a colony of healthy CV-1 green monkey kidney cells that have been exposed to crystal violet but not fluorescent light. The left half of the panel in Figure 1(b) shows a colony of healthy CV-1 green monkey kidney cells that have been exposed to crystal violet and fluorescent light. The data of Figure 1(b) demonstrates that crystal violet has a negligible phototoxic effect on healthy CV-1 green monkey kidney cells when irradiated with fluorescent light having a wavelength appropriate to produce phototoxic effects on HT-29 human colon adenocarcinoma cells. The data in Figures 1(a) and (b) together demonstrate the selective phototoxicity of crystal violet towards human colon adenocarcinoma cells (HT-29 cells) relative to non-cancerous green monkey kidney cells (CV-1 cells).

1A



1B

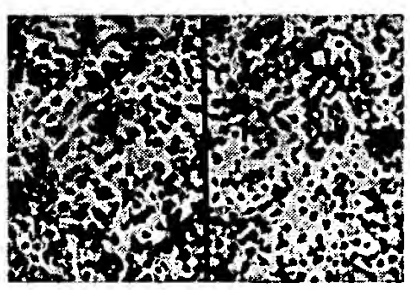


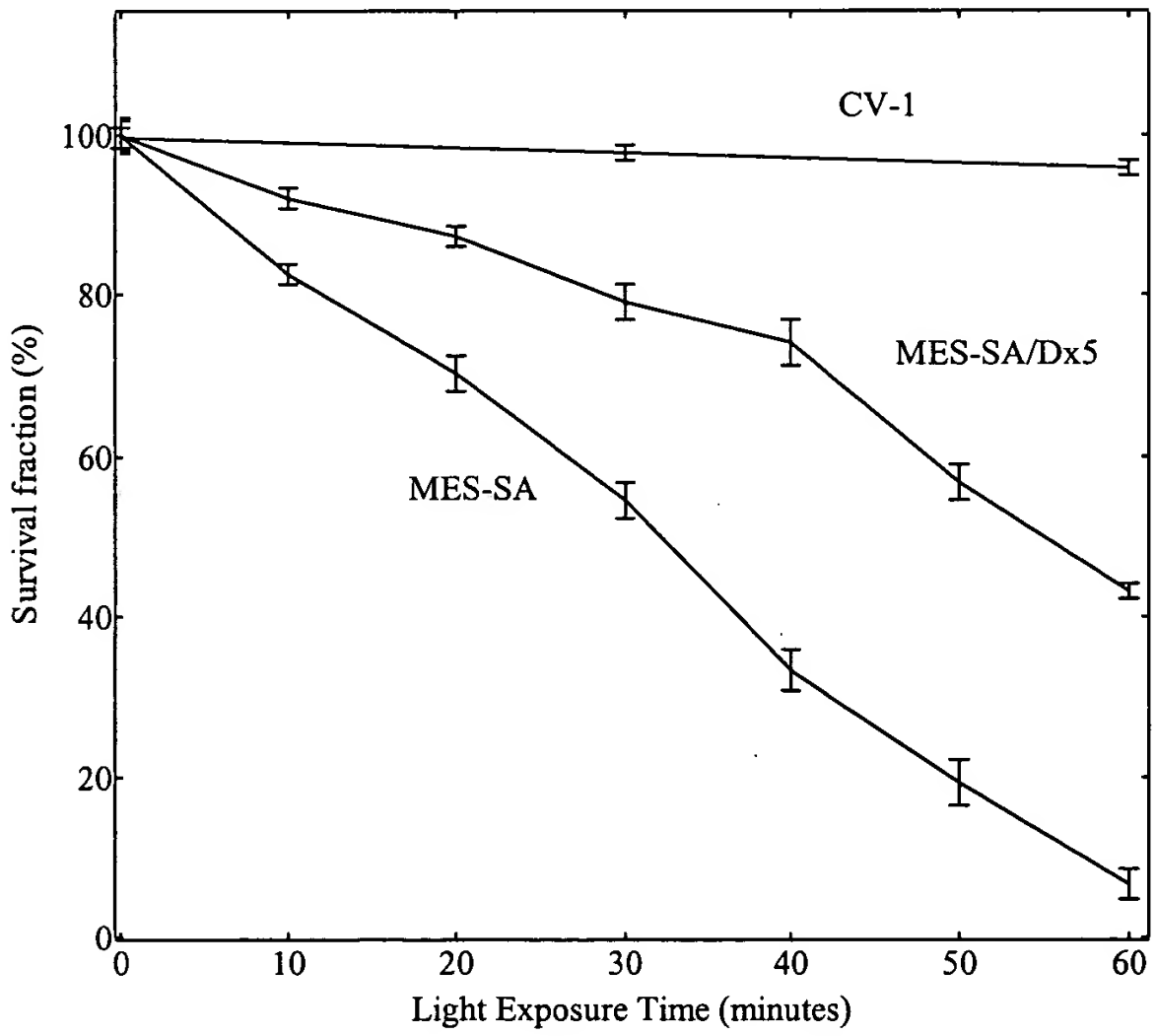
Figure 1. Phototoxicity of crystal violet towards tumor (panel 1A) and normal (panel 1B) cells. Tumor (HT-29) and normal (CV-1) cells were incubated for 1 hour with 5 μM

crystal violet, washed, and exposed to fluorescent light for 1 hour as described in section 8 (see below). Twenty four hours after light exposure the cells were fixed with methanol and stained. Before light exposure, half of each Petri dish was masked with black tape. The area protected from light is represented by the right-half of each panel. Upon light exposure, only the HT-29 cells were heavily destroyed. No toxic effects were observed against cells kept in the dark, or against cells exposed to light but not contacted with crystal violet.

8. The experiments used to obtain Figures 1(a) and (b) were conducted as follows: HT-29 human colon adenocarcinoma cells (ATCC HTB-38) and CV-1 green monkey kidney cells (ATCC CCL-70) were obtained from the American Type Culture Collection. Both cell lines were cultured separately using standard techniques in RPMI 1640 media supplemented with 10% FBS, amphotericin B (0.63 μg/ml), penicillin (200 unit/ml), and streptomycin (200 μg/ml). Small petri dishes were seeded at low cell densities (9×10^5 cells per 3 ml of growth media) to permit formation of evenly distributed monolayers and the cells were allowed to attach overnight. Before irradiation, the cells were washed twice fresh Dulbecco's phosphate-buffered saline (DPBS) solution and subsequently incubated for 1 hour at 37°C in the presence of 3 ml of 5 μM crystal violet dye solution prepared in fresh phosphate buffer saline (PBS). The original crystal violet stock solutions were prepared in ethanol, and the final ethanol concentration was always kept below the 1% level. The cells were then washed twice with DPBS and re-incubated with 3 ml of PBS. Prior to light exposure, half of each petri dish was masked with black tape. The cells were then exposed to fluorescent light to selectively destroy the HT-29 cells. During light exposure, the cells were kept at approximately 20 cm from a bank of cold fluorescent lamps (F40 CWP fluorescent lamp from Sylvania) for sixty minutes at room temperature. After irradiation, the PBS solution present in the petri dishes was replaced by fresh growth media and

the cells were incubated for 24 hours before being fixed with methanol and stained with a 0.1% aqueous crystal violet solution.

9. Graph I, shown below, shows data obtained by Parthiv N. Vora, a graduate student in my research group, under my direct supervision. The data in graph I show the fraction of surviving MES-SA human uterine cancer cells, MES-SA/Dx5 multiple drug resistant human uterine cancer cells, and normal healthy CV-1 cells that have been exposed to crystal violet and fluorescent light as a function of light exposure time. The data in Graph I demonstrates the selective phototoxicity of crystal violet toward human uterine sarcoma cells and multiple drug resistant mutant human uterine sarcoma cells relative to non-cancerous CV-1 cells.



Graph I. Phototoxic effects of Crystal Violet against normal cells (CV-1, solid line), multiple drug resistant human uterine sarcoma cells (MES-SA/Dx5, dotted line), and human uterine sarcoma cells (MES-SA, dashed line) as a function of light exposure time. The

cells were incubated for 1 hour in the presence of 5 micromolar Crystal Violet and subsequently illuminated with a bank of cold fluorescence lamps (fluence rate = 18 W/square meter). Each data point represents the average of three independent measurements (the respective standard deviations –error bars- are shown). No toxic effects were observed against cells kept in the dark, or against cells illuminated but not exposed to Crystal Violet.

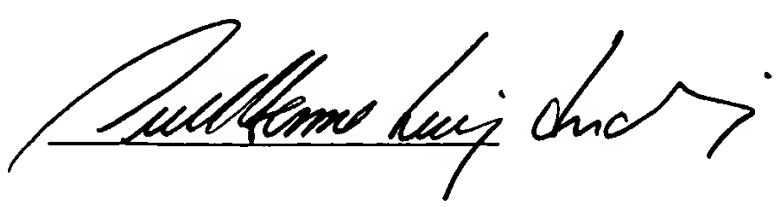
10. The experimental conditions used to obtain the data shown in Graph I were analogous to those used to obtain the data shown in Figure 1. The experiments shown in Graph I were conducted as follows: human uterine sarcoma cells (MES-SA) and multiple drug resistant human uterine sarcoma cells (MES-SA/Dx5) were obtained from the American Type Culture Collection. Both cells lines were cultured separately in MEM α media supplemented with 10% FBS, penicillin (200 units/ml) and streptomycin (200 mg/ml). CV-1 cells were grown as previously described in section 8 of this declaration. Small Petri dishes (35 mm in diameter) were seeded at low cell densities (9×10^5 cells per 3 ml of growth media) to provide for the formation of evenly distributed monolayers, and the cells allowed to attach overnight. Before light exposure, the petri dishes containing either MES-SA, MES-SA/Dx5 or CV-1 cells (only one cell type per dish) were washed twice with fresh PBS solution and subsequently incubated for 60 minutes in the presence of 3 ml of 5 μM crystal violet solution prepared in fresh PBS. The original crystal violet stock solution was prepared in ethanol, and the final ethanol content in the PBS solutions used for cell incubation was always kept at the 1% level. After incubation for 60 minutes in the dark, the cells were immediately exposed to light. During light exposure, the cells were kept at room temperature and at approximately 20 cm from the bank of cold fluorescent lamps. After light exposure, the PBS solution present in the petri dishes was replaced by fresh growth media and the cells incubated for 24 hours. After this final 24 hours

incubation in fresh growth media, the cells were detached from the culture dishes with trypsin/EDTA in PDS and the survival fraction measured using the trypan blue dye exclusion test.

11. The basic methodology used to obtain the data for human colon adenocarcinoma cells, presented in Figure 1, and the data for human uterine cancer cells, presented in Graph I, is the same as that used to obtain the data for leukemia cells, presented in the Application. The differences between the experimental protocols described in sections 8 and 10 of this declaration and the experimental protocols described in the Application represent only small variations in the methodology presented in the Application. A person having ordinary skill in the art of photodynamic therapy would be able to practice the invention described in the Application to selectively destroy human adenocarcinoma cells and human uterine cancer cells.

12. Taken together, the data presented in the Application, Figure 1, and Graph I demonstrate that the methods for selectively killing cancer cells recited in claims 6-10 and described in the specification of the Application are generally applicable to and suitable for the selective destruction of broad variety of cancer cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 09/23/02 Signature: 

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Education

BS, Chemistry, State University of Campinas, Brazil, 1979.

Ph.D., Physical Chemistry, University of São Paulo, Brazil, 1988.

Experience

02/95-present	Assistant Professor, University of Wisconsin at Madison, School of Pharmacy.
12/91-01/95	Postdoctoral Research Associate, Boston University, Department of Chemistry.
05/89-11/91	Postdoctoral Research Associate, Harvard University, Departments of Molecular & Cellular Biology and Chemistry.
12/88-04/89	FAPESP Fellow, University of São Paulo, Institute of Chemistry.
07/86-11/86	DAAD Fellow, University of Düsseldorf, Department of Physiological Chemistry, Medical School.
07/83-11/88	Research Assistant, Ph.D. Program, University of São Paulo, Institute of Chemistry.
03/83-06/85	Community College Teacher, Oswaldo Cruz College and São Bernardo do Campo College, São Paulo, Brazil.
07/78-06/83	High School Teacher, São Paulo State High Schools, Brazil.
07/77-06/78	Undergraduate Research Assistant, University of Campinas, Department of Chemistry.

Honors and Awards

Graduate School Fellowship, Foundation for the Promotion of Science of the State of São Paulo (FAPESP, São Paulo, Brazil).

Visiting Graduate Student Scholarship, German Academic Exchange Service (DAAD, Bonn, Germany).

Postdoctoral Fellowships, National Council for Scientific and Technological Development (CNPq; Brasilia, Brazil), and Foundation for the Promotion of Science of the State of São Paulo (FAPESP, São Paulo, Brazil).

Recognition Award, Wisconsin Youth Apprenticeship Program, Dane County Area, 1997.

Professional Societies

American Association of Colleges of Pharmacy, American Association of Pharmaceutical Scientists, American Chemical Society, American Society for Photobiology, Biophysical Society, Inter-American Photochemical Society, International Union of Pure and Applied Chemistry, Rho Chi National Honor Society.

Review Panels

US Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (Ad hoc reviewer)

Chemical Physics Letters

Journal of Organic Chemistry

Journal of the Brazilian Chemical Society

Lasers in Surgery and Medicine

Marine Chemistry

Organic Letters

Photochemistry and Photobiology

Publications

23. Kandela, I. K.; Bartlett, J. A.; Indig, G. L. (2002) *"Effect of Molecular Structure on the Selective Phototoxicity of Triarylmethane Dyes Towards Tumor Cells."* Photochemical and Photobiological Sciences, 1, 309-314.

22. Oliveira, C. S.; Branco, K. P.; Baptista, M. S.; Indig, G. L. (2002) *"Solvent and Concentration Effects on the Visible Spectra of Tri-Para-Dialkylamino-Substituted Triarylmethane Dyes in Liquid Solutions."* Spectrochimica Acta A, in press.

21. Lewis, L. M.; Indig, G. L. (2002). *"Effect of Dye Aggregation on Triarylmethane-Mediated Photoinduced Damage of Hexokinase and DNA."* Journal of Photochemisttry and Photobiology B: Biology, 67, 139-148.

20. Lewis, L. M.; Indig, G. L. (2001). *"Photonuclease Activity of Mitochondrial Triarylmethane Photosensitizers."* Pharmaceutical and Pharmacological Letters, 11, 22-25.

19. Indig, G. L.; Anderson, G. S.; Nichols, M. G.; Bartlett, J. A.; Mellon, W. S.; Sieber, F. (2000). *"Effect of Molecular Structure on the Performance of Triarylmethane Dyes as Therapeutic Agents for Photochemical Purging of Autologous Bone Marrow Grafts from Residual Tumor Cells."* Journal of Pharmaceutical Sciences, 89, 88-99.

18. Lewis, L. M.; Indig, G. L. (2000). *"Solvent Effects on the Spectroscopic Properties of Triarylmethane Dyes."* Dyes and Pigments, 46, 145-154.

17. Kowaltowski, A. J.; Turin, J.; Indig, G. L.; Vercesi, A. E. (1999). *"Mitochondrial Effects of Triarylmethane Dyes."* Journal of Bioenergetics and Biomembranes, 31, 581-590.

16. Bartlett, J. A.; Indig, G. L. (1999). *"Effect of Self-Association and Protein Binding on the Photochemical Reactivity of Triarylmethanes. Implications of Noncovalent Interactions on the Competition between Photosensitization Mechanisms Type I and Type II."* Photochemistry and Photobiology, 70, 490-498.

15. Bartlett, J. A.; Indig, G. L. (1999). *"Spectroscopic and Photochemical Properties of Malachite Green Noncovalently Bound to Bovine Serum Albumin."* Dyes and Pigments, 43, 219-226.

14. Indig, G. L. (1999). *"Mechanisms of Action of Cationic Dyes in Photodynamic Therapy of Tumors."* Recent Research Developments in Pure and Applied Chemistry, 3, 9-19.

13. Baptista, M. S.; Indig, G. L. (1998). *"Effect of BSA-Binding on Photophysical and Photochemical Properties of Triarylmethane Dyes."* Journal of Physical Chemistry, 102B, 4678-4688.

12. Baptista, M. S.; Indig, G. L. (1997). *"Mechanism of Photobleaching of Ethyl Violet Non-Covalently Bound to Bovine Serum Albumin."* Chemical Communications, 18, 1791-1792.

11. Indig, G. L. (1997). *"Mechanism of Dye Bleaching upon Laser Excitation of Crystal Violet Bound to Bovine Serum Albumin."* Chemistry Letters, 3, 243-244.

10. Indig, G.L. (1996). *"Photochemistry of Triarylmethane Dyes Bound to Proteins."* Proceedings of the International Society for Optical Engineering, 2675, 228-237.

9. Jones, II, G.; Indig, G. L. (1996). *"Spectroscopic and Chemical Binding Properties of Humic Acids in Water."* New Journal of Chemistry, 20, 221-232.

8. Jones, II, G.; Oh, C.; Indig, G. L. (1994). *"Organic Dyes Bound to Polyelectrolytes: Photophysical Probes of Binding Domains and Biopolymer Conformation."* In Environmental Aspects of Surface and Aquatic Photochemistry, Crosby, D.; Zepp, R.; Held, G. R., Eds., Lewis Publishers: Chelsea, Michigan, 129-136.

7. Indig, G. L.; Jay, D. G.; Grabowski, J. J. (1992). *"The Efficiency of Malachite Green, Free and Protein Bound, as a Photon-to-Heat Converter."* Biophysical Journal, 61, 631-638.

6. Indig, G. L.; Catalani, L. H.; Wilson, T. (1992). *"Quenching of Triplet Acetone by Mesitylene or Durene: Energy Transfer or Exciplex Formation?"* Journal of Physical Chemistry, 96, 8967-8973.

5. Indig, G. L.; Wilson, T. (1992). *"Triplet Lifetime and Concentration Quenching of Methylbenzenes in Solution."* Journal of Photochemistry and Photobiology A: Chemistry, 63, 195-199.

4. Indig, G. L.; Campa, A.; Bechara, E. J. H.; Cilento, G. (1989). *"Triplet Acetone Can Initiate Lipid Peroxidation".* In Medical, Biochemical and Chemical Aspects of Free Radicals, Hayaishi, O.; Niki, E.; Kondo, M.; Yoshikawa, T., Eds., Elsevier: Amsterdam, 981-984.

3. Villablanca, M.; Indig, G. L.; Slawinska, D.; Slawinski, J. (1989). *"Studies on Chemiluminescence in the Enzymatic and Autoxidative Transformation of 3,4-Dihydroxyphenylalanine into Eumelanins."* Journal of Bioluminescence and Chemiluminescence, 3, 181-190.

2. Indig, G. L.; Campa, A.; Bechara, E. J. H.; Cilento, G. (1988). *"Conjugated Diene Formation Promoted by Triplet Acetone Acting Upon Arachidonic Acid."* Photochemistry and Photobiology, 48, 719-723.

1. Indig, G. L.; Cilento, G. (1987). *"Peroxidase-Promoted Aerobic Oxidation of 2-Nitropropane: Mechanism of Excited State Formation."* Biochimica et Biophysica Acta, 923, 347-354.

Abstracts and Conference Presentations

76. Indig, G. L.; Kandela, I. K.; Lee, W. (2002).*"Effect of Molecular Structure on the Phototoxicity of Triarylmethane Dyes Towards Tumor and Normal Cells"* The 30th Annual Meeting of the American Society for Photobiology,, Quebec City, Canada, in press.

75. Indig, G. L.; Bartlett, J. A.; Lewis, L. M.; Kandela, I. K. (2001). *"Chemical Aspects of Mitochondrial Targeting in Photochemotherapy."* XII Inter-American Photochemical Society Conference, Cordoba, Argentina, Book of Abstracts, XLII-XLIII.

74. Indig, G. L.; Bartlett, J. A.; Lewis, L. M. (2001). *"Molecular Exciton Effects on Photophysical and Photochemical Properties of Biopolymer-Dye Complexes."* Inter-American Workshop on Photochemistry, Photophysics and Spectroscopy in Organized Media", Cordoba, Argentina, Book of Abstracts, 31 (abstract L20).

73. Indig, G. L. (2001). *"Mitochondrial Targeting in Photodynamic Therapy: A Novel Therapeutic Strategy that Provides for Highly Selective Destruction of Tumor Cells and Minimizes the Role of Oxygen on Photosensitization."* The 4th International Meeting on Photostability of Drugs and Drug Products, Research Triangle Park, NC, Conference Book, Technical Session 1.

72. Indig, G. L. (2001). *"Basic Principles in Pharmaceutical Photostability Testing."* Short Course on Photostability of Drugs and Drug Products, The 4th International Meeting on Photostability of Drugs and Drug Products, Research Triangle Park, NC, Course Book, Lecture 1.

71. Indig, G. L., Bartlett, J. A., Lewis, L. M. (2001). *"Effect of Self-Association and Biopolymer Binding on the Photonuclease/Photoprotease Activity of Triarylmethane Photosensitizers."* The 29th Annual Meeting of the American Society for Photobiology,, Chicago, IL, Book of Abstracts, 89 (abstract 269).

70. Oliveira, C. S.; Indig, G. L.; Baptista, M. S. (2001). *"Photophysical and Photochemical Properties of Crystal Violet in Reversed Micelles."* XII Inter-American Photochemical Society Conference, Cordoba, Argentina, Book of Abstracts, 68.

69. Bartlett, J. A.; Indig, G. L. (2001). *"Enzymatic Synthesis of New Cationic Photosensitizers."* The 29th Annual Meeting of the American Society for Photobiology, Chicago, IL, Book of Abstracts, 46 (abstract 138).

68. Branco, K. P.; Oliveira, C. S.; Indig, G. L.; Baptista, M. S. (2001). *"Characterization of Triarylmethane Dimers and Ion-Pairs in Solution and in Restricted Reaction Spaces."* The 30th Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology, Caxambu, Brazil, Book of Abstracts, abstract Q-10.

67. Oliveira, C. S.; Indig, G. L.; Baptista, M. S. (2001). *"Spectroscopic Characterization of the Spatial Distribution of Crystal Violet in Reversed Micelles."* The 30th Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology, Caxambu, Brazil, Book of Abstracts, abstract Q-09.

66. Kandela, I. K.; Bartlett, J. A.; Mellon, W. S.; Indig, G. L. (2001) *"New Cationic Photosensitizers for the Selective Destruction of Tumor Cells Via Mitochondrial Targeting."* The 29th Annual Meeting of the American Society for Photobiology, Chicago, IL, Book of Abstracts, 46 (abstract 139).

65. Indig, G. L. (2001). *"Immune Cell Modulation and Treatment of Autoimmune Diseases with Red and Near Infra-Red Light."*, Pharma-Transfer, February, Abstract ID M0587.

64. Bartlett, J. A.; Indig, G. L. (2001). *"Mechanisms of Action of Crystal Violet in Photodynamic Therapy."* The 33rd Pharmaceutics Graduate Student Research Meeting, Madison, WI, Book of Abstracts, 123 (abstract PM02).

63. Lewis, L. M.; Indig, G. L. (2001). *"Spectroscopic and Photochemical Properties of Photosensitizer-LDL Complexes."* The 33rd Pharmaceutics Graduate Student Research Meeting, Madison, WI, Book of Abstracts, 138 (abstract PM17).

62. Kandela, I. K.; Bartlett, J. A.; Indig, G. L. (2001) *"New Triarylmethane Photosensitizers for the Selective Destruction of Tumor Cells Via Mitochondrial Targeting."* The 33rd Pharmaceutics Graduate Student Research Meeting, Madison, WI, Book of abstracts, 89 (abstract PS37).

61. Lewis, L. M.; Indig, G. L. (2000). *"Photoinduced Inactivation of Hexokinase and DNA by Triarylmethane Dyes."* The XVIII IUPAC Symposium on Photochemistry, Dresden, Germany, Book of Abstracts, 308-309.

60. Lewis, L. M.; Bartlett, J. A.; Amin, K.; Indig, G. L. (2000). *"Mechanisms of Phototoxicity of Triarylmethane Dyes Towards Model Mitochondrial Targets."* The 13th International Congress on Photobiology, San Francisco, CA, Book of Abstracts, 29 (abstract 89).

59. Bartlett, J. A.; Indig, G. L. (2000). *"Effect of Transition Dipole Moment Orientation on the Photoreactivity of Dye Aggregates."* The 13th International Congress on Photobiology, San Francisco, CA, Book of Abstracts, 29 (abstract 90).

58. Indig, G. L.; Anderson, G. S.; Nichols, M. G.; Bartlett, J. A.; Mellon, W. S.; Sieber, F. (2000). *"Triarylmethane Dyes as Therapeutic Agents for the Photochemical Purging of Autologous Bone Marrow Grafts from Residual Tumor Cells. "* The 44th Annual Meeting of the Biophysical Society, New Orleans, LA, Biophysical Journal, 78, 256A (abstract 1515).

57. Indig, G. L. (2000). *"Mitochondrial Targeting in Photochemotherapy: A Novel Therapeutic Strategy."* Pharma-Transfer, July, Abstract ID 588699.

56. Indig, G. L. (2000). *" Long-Wavelength Absorbing Photoresponsive Polymer Gels."*, Pharma-Transfer, August, Abstract ID AL0029.

55. Bartlett, J. A.; Indig, G. L. (2000). *"Synthesis Of New Triarylmethane Dyes Via HRP-Catalyzed Oxidations."* Annual Meeting of the American Association of Pharmaceutical Scientists, Indianapolis, IN, CD of Abstracts, abstract 3234.

54. Lewis, L. M.; Indig, G. L. (2000). *"Photoinactivation of Model Mitochondrial Targets by Triarylmethane Dyes."* Annual Meeting of the American Association of Pharmaceutical Scientists, Indianapolis, IN, CD of Abstracts, abstract 3302.

53. Bartlett, J. A.; Indig, G. L. (2000). *"Enzymatic Synthesis of New Cationic Triarylmethane Dyes."* The 30th Pharmaceutics Graduate Student Research Meeting, Columbus, OH, Book of Abstracts, 99 (abstract PS 65).

52. Lewis, L. M.; Indig, G. L. (2000). *"Photoinactivation of Model Subcellular Targets by Triarylmethane Dyes."* The 30th Pharmaceutics Graduate Student Research Meeting, Columbus, OH, Book of Abstracts, 105 (abstract PS 71).

51. Indig, G. L.; Anderson, G. S.; Nichols, M. G.; Bartlett, J. A.; Mellon, W. S.; Sieber, F. (2000). *"Triarylmethane Dyes as Therapeutic Agents for the Photochemical Purging of Autologous Bone Marrow Grafts from Residual Tumor Cells."* Workshop on Emerging Techniques in Screening and Imaging Sciences, Madison, WI.

50. Indig, G. L.; Anderson, G. S.; Nichols, M. G.; Bartlett, J. A.; Mellon, W. S.; Sieber, F. (1999). *"Assessment of Crystal Violet and Other Triarylmethane Dyes as Photosensitizers for Purging of Autologous Bone Marrow Grafts from Residual Tumor Cells."* Gordon Conference on Organic Photochemistry, New London, CT.

49. Bartlett, J. A.; Indig, G. L. (1999). *"Effect of Self-Association on the Photochemical Reactivity of Triarylmethane Dyes in Aqueous Solution."* The 31st Pharmaceutics Graduate Student Research Meeting, Kansas City, MO, Book of Abstracts, abstract PS-19.

48. Lewis, L. M.; Indig, G. L. (1999). *"Characterization of Noncovalent Complexes of Triatylmethane Dyes and Low Density Lipoprotein."* The 31st Pharmaceutics Graduate Student Research Meeting, Kansas City, MO, Book of Abstracts, abstract PS-30.

47. Indig, G. L., Bartlett, J. A.; Lewis, L. M. (1999). *"Effect of Self-Association and Protein Binding on the Photoreactivity of Triarylmethane Dyes."* The 27st Annual Meeting of the American Society for Photobiology, Washington, DC, Photochemistry and Photobiology, 69(S), 78 (abstract WPM-E10).

46. Indig, G. L. (1998). *"Photosensitization in Restricted Reaction Spaces: Effect of Protein Binding on Mechanisms of Action of Triarylmethanes."* International Meeting on Singlet Molecular Oxygen. Chemical, Biological and Medical Aspects, Caraguatatuba, São Paulo, Brazil, Book of Abstracts, pp 26-27.

45. Bartlett, J. A.; Indig, G. L. (1998). *"Effect of Self-Association on the Photochemical Reactivity of Triarylmethane Dyes in Aqueous Solution."* International Meeting on Singlet Molecular Oxygen. Chemical, Biological and Medical Aspects, Caraguatatuba, São Paulo, Brazil, Book of Abstracts, p 68.

44. Jimenez, J. A. C.; Jones, G. II; Indig, G. L. (1998). *"Interaction of Organic Dyes with Humic Acids."* The 216th National Meeting of the American Chemical Society, Boston, MA, Division of Physical Chemistry, Book of Abstracts, abstract 315.

43. Indig, G. L.; Lewis, L. M.; Baptista, M. S.; Amin, K. (1998). *"Mechanisms of Photosensitization in Restricted Reaction Spaces. Effect of Protein Binding on the Photoreactivity of Triarylmethanes."* The 26st Annual Meeting of the American Society for Photobiology, Snowbird, UT, Photochemistry and Photobiology, 67(S), 94 (abstract WPM-E4).

42. Lewis, L. M.; Indig, G. L. (1998). *"Binding of Triarylmethane Dyes to Human Low Density Lipoproteins."* The 31st Great Lakes Regional Meeting of the American Chemical Society, Milwaukee, WI, Book of Abstracts, abstract 281.

41. Amin, K.; Indig, G. L. (1998). *"Mechanisms of Photoinactivation of Enzymes Mediated by Ethyl Violet."* The 31st Great Lakes Regional Meeting of the American Chemical Society, Milwaukee, WI, Book of Abstracts, abstract 280.

40. Bartlett, J. A.; Indig, G. L. (1998). *"Photophysics of N-N'-Bis(2-ethyl-1,3-Dioxolane) Kryptocyanine in Solution and in Glass of Sucrose Octaacetate."* The 31st Great Lakes Regional Meeting of the American Chemical Society, Milwaukee, WI, Book of Abstracts, abstract 279.

39. Amin, K.; Baptista, M. S.; Indig, G. L. (1998). *"Mechanisms of Photoinactivation of Enzymes Mediated by Triarylmethane Dyes."* The 42nd Annual Meeting of the Biophysical Society, Kansas City, MO, Biophysical Journal, 74(2), A367 (abstract Th-Pos121).

38. Amin, K.; Indig, G. L. (1997). *"Bleaching of Triarylmethane Dyes Promoted by Horseradish Peroxidase."* The XVII Midwest Enzyme Chemistry Conference, Chicago, IL, Book of Abstracts, p. 43.

37. Baptista, M. S.; Indig, G. L. (1997). *"Effect of BSA-Binding on Photochemical Properties of Triarylmethane Dyes."* Gordon Conference on Organic Photochemistry, Newport, RI.

36. Indig, G. L.; Lewis, L. M; Baptista, M. S. (1997). *"Spectroscopic and Photochemical Properties of Victoria Blue Dyes and Other Triarylmethanes Bound to Serum Proteins."* The 25 th Annual Meeting of the American Society for Photobiology, St. Louis, MO, Photochemistry and Photobiology 65(S), 24 (abstract SPM-G11).

35. Ehlert, D. A.; Kavalauskas, M. P.; Indig, G. L. (1997). *"Entrapment of Styryl-7 into the Cavity of Horse Spleen Apoferritin."* The 41st Annual Meeting of the Biophysical Society, New Orleans, LA, Biophysical Journal, 72(2), A316 (abstract W-Pos390).

34. Anderson, G. B.; Indig, G. L. (1996). *"Photoreactivity of Crystal Violet Bound to Low Density Lipoproteins."* The 212th National Meeting of the American Chemical Society, Orlando, FL, Division of Physical Chemistry, Book of Abstracts, abstract 275.

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